Drug stability testing results and conclusion

Research unit: Chinese Academy of Medical Sciences
Cancer Research Institute

ACAPHA Stability Research

ACAPHA is prepared from 6 herbal ingredient Dioscorea bubifera, Polygonum bistora, Sophora tonkinensis, Prunella vulgaris, Sonchus brachyotus and Distamnus dasycarpus. The main usage is for the prevention of esophageal cancer. According to the requirement of "New Drug Submission for Approval", we have researched on the stability of the ACAPHA tablets.

1. Research Method:

According to new drug(Chinese medicine) stability assessment supplementary requirement related to the stability testing of ACAPHA tablets, we adopted the method of storage of specimens in warm room for evaluation. Under warm room environment, 3 lots of ACAPHA were stored and tested during a 2-year period. The assessment items and methods are as follows:

1. Physical nature: visual assessment, mainly looking for fungal spots, color spots and abnormal coloration.
2. Identification: based on quality standard assessment protocol. Positive response is noted as "+".
3. Content evaluation: refer to method of quality standard.
4. Hardness: Due to the fact that Chinese Pharmacopoeia has no consistent standard, we therefore based our testing on published reports and information in the manual of the tablet testing instrument. An average hardness in kg was obtained for 10 tablets.
5. Dissolution time: this was based on the testing protocol described in the Chinese Pharmacies. Dissolution time should be less that 60 minutes.
6. Hygiene testing: this was based on the testing protocol described in the Chinese Pharmacopoeia. The results should comply with the drug hygiene standard and related regulation(86) section. The drug in the tablets should contain less than 10,000 E. Coli and bacterial counts and less than 500 fungal counts per g of sample.

2. Research Results:

Results of testing of 3 batches of tablets are reported in tables 1-3. In the tablets, Identification #1 is based on TLC identification of Sophora tonkinesis and Identification #2 is based on TLC identification of Dictamnus dasycarpus. Based on the TLC results of these two herbs the 3 lots of ACAPHA showed that they have the same color spots detected at the same locations in tablets before and storage for 2 years. There was not obvious abnormal spots observed.

3. Conclusion:

Adopting warm room storage of specimens for assessment, we assessed 3 lots of ACAPHA for a period of 2 years. The assessment results indicate that ACAPHA's physical condition identification, content, hardness, dissolution time and hygiene standards all fulfilled quality standard. This study confirmed the quality stability of the product.

Table 1. ACAPHA (Approval Lot # 880125) Stability Assessment .

Storage Duration / Assessment		0 month	3 month	6 month	9 month	12 month	18 month	24 mon
Physical nature		Normal	Normal	Normal	Normal	Normal	Normal	Normal
Identification	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
Content (mg/tablet)		0. 6329	0. 6649	0. 6556	0. 6674	0. 6533	0. 6617	0. 6558
Hardness (Kg)		6. 6	6. 3	7. 4	6. 8	6. 5	7. 2	7. 0
Dissolution time (Min.)		50	56	54	48	52	46	47
Hygiene Testing	E.coli count (#/g)	0	0	0	0	0	0	0
	Bacterial count (#/g)	1200	1150	1186	1432	1320	1068	1246
	Fungal count (#/g)	68	74	66	70	74	64	72

Table 2. ACAPHA (Approval Lot # 880208) Stability Assessment .

Storage Duration / Assessment		0 month	3 month	6 month	9 month	12 month	18 month	24 month
Physical nature		Normal	Normal	Normal	Normal	Normal	Normal	Normal
Identification	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
Content (mg/tablet))		0. 6399	0. 6234	0. 6373	0. 6341	0. 6459	0. 6387	0. 6431
Hardness (Kg)		5. 7	6. 2	5. 5	6. 0	5. 4	5. 5	5. 9
Dissolution time (Min.)		44	42	45	48	45	43	47
Hygiene Testing	E.coli count (#/g)	0	0	0	0	0	0	0
	Bacterial count (#/g)	2046	1980	2352	2004	1864	2228	2118
	Fungal count (#/g)	102	124	98	106	126	94	94

Table 3. ACAPHA (Approval Lot # 880211) Stability Assessment .

Storage Duration / Assessment		0 month	3 month	6 month	9 month	12 month	18 month	24 month
Physical nature		Normal	Normal	Normal	Normal	Normal	Normal	Normal
Identification	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
Content (mg/tablet)		0. 6371	0. 6612	0. 6535	0. 6588	0. 6613	0. 6556	0. 6612
Hardness (Kg)		5. 2	5. 0	5. 2	4. 9	4. 8	4. 9	5. 2
Dissolution time (Min.)		43	42	42	41	44	46	45
Hygiene Testing	E.coli count (#/g)	0	0	0	0	0	0	0
	Bacterial count (#/g)	842	792	834	866	790	808	800
	Fungal count (#/g)	92	88	86	90	92	94	98

资料编号8

药物的稳定性试验资料、结论

研究单位　　中国医学科学院　　中国研究所

增生平片的稳定性研究

增生平片由黄药子、拳参、山豆根、夏枯草、北败酱和白鲜皮六味药物组成，主要用于预防食管癌。根据《新药审批办法》的要求，我们对其进行了稳定性研究。

一．实验方法：

根据新药（中药）稳定性试验资料的补充规定中关于片剂稳定性试验要求，我们采用室温留样考察法，在室温条件下，对增生平片三批样品，进行了为期二年的稳定性试验。考察项目与方法如下：

1.性状：感官检查，主要观察有无霉点、色斑等异常情况。

2.鉴别　　：按质量标准草案的检测方法进行测定，正反应记作“＋”。

3.含量测定　　：方法见质量标准。

4.硬度：由于《中国药典》未做统一规定，我们参照文献　及片剂四用测定仪说明书进行测定。取本品10片，分别测定其硬度（公斤），求平均值，即得。

5.崩解时限：按《中国药典》规定的方法测试。崩解时限应在60分钟以内

6.卫生学检查：按《中国药典》规定的方法检查，应符合(86)部颁　药品卫生标准》的有关规定。即含原药材的片剂，每克样品不得检出大肠杆菌，细菌数不得超过10,000个，霉菌数不得超过 500个。

二．实验结果：

三批样品的考察结果分别见表1，表2和表3。表中的鉴别4为山豆根的薄层层析。鉴别5为白鲜皮的薄层层析。以上两种薄层层析结果表明，在对照药材的相应位置，三批样品均显示相同颜色的斑点，而且在放置两两年以后，未出现明显的异常斑点。

三．小结：

采用室温留样考察法，对增生平片三批样品，进行了为期二年的稳定性试验考察。考察结果表明，增生平片的性状、鉴别、含量、硬度、崩解时限和卫生学检查等项指标均符合质量标准，说明该品质量稳定。

表1 增生平片（批号88 0125）稳定性考察表

考察项目 \ 贮存时间		0 月	3 月	6 月	9 月	12 月	18 月	24 月
性状		无异常	无异常	无异常	无异常	无异常	无异常	无异常
鉴别	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
含量（苦参碱 mg/片）		0.6329	0.6649	0.6556	0.6674	0.6533	0.6617	0.6558
硬度（Kg）		6.6	6.3	7.4	6.8	6.5	7.2	7.0
崩解时限（分钟）		50	56	54	48	52	46	47
卫生学检查	大肠杆菌（个/g）	0	0	0	0	0	0	0
	细菌（个/g）	1200	1150	1186	1432	1320	1068	1246
	霉菌（个/g）	68	74	66	70	74	64	72

表2 增生平片（批号 880208）稳定性考察表

考察项目 \ 贮存时间		0 月	3 月	6 月	9 月	12 月	18 月	24 月
性状		无异常	无异常	无异常	无异常	无异常	无异常	无异常
鉴别	1	+	+	+	−	+	+	−
	2	+	+	+	−	+	+	−
含量（苦参碱 mg/片）		0.6399	0.6234	0.6373	0.6341	0.6459	0.6387	0.643[illegible]
硬度（Kg）		5.7	6.2	5.5	6.0	5.4	5.5	5.9
崩解时限（分钟）		44	42	45	48	45	43	47
卫生学检查	大肠杆菌（个/g）	0	0	0	0	0	0	0
	细菌（个/g）	2046	1980	2352	2004	1864	2228	2116
	霉菌（个/g）	102	124	98	106	126	94	94

表3 增生平片（批号 880211）稳定性考察表

考察项目 \ 贮存时间		0 月	3 月	6 月	9 月	12 月	18 月	24 月
性状		无异常	无异常	无异常	无异常	无异常	无异常	无异常
鉴别	1	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+
含量(苦参碱 mg/片)		0.6371	0.6612	0.6535	0.6588	0.6613	0.6556	0.6612
硬度 (Kg)		5.2	5.0	5.2	4.9	4.8	4.9	5.2
崩解时限(分钟)		43	42	42	41	44	46	45
卫生学检查	大肠杆菌(个/g)	0	0	0	0	0	0	0
	细菌(个/g)	842	792	834	866	790	808	800
	霉菌(个/g)	92	88	86	90	92	94	98

Batch Consistency and Stability

Performed by BCIT

FORENSIC SCIENCE CENTRE

Herbal Evaluation and Analysis Laboratory

May 2002 **HPLC Profiling of ACAPHA Extracts**

Collaborators **Dr. Joseph Tai & Global Cancer Strategies**

Sampling Information & Testing Protocol

ACAPHA samples were received from Dr. Joseph Tai on May 2, 2002. The samples consisted of ACAPHA powders. All samples were identified by a unique tag number. The ACAPHA samples were received by Paula Brown for the Herbal Evaluation & Analysis Laboratory of the Forensic Sciences Centre. Each sample was logged in as a test sample according to the standard operating procedures (HEAL-SOP-002), and assigned a unique identification code. The sample preparation and instrumental analysis was conducted by Hazrah Moothoo under the supervision of Paula Brown, Principal Scientist, Herbal Evaluation & Analysis Laboratory, BCIT. The details of the sample preparation and HPLC methodology are as per HEAL Analytical Test Method, BCIT-ATM-001-00. The unused samples were retained in the original sample packaging, stored at room temperature and protected from direct light.

Analysis of HPLC Profiles

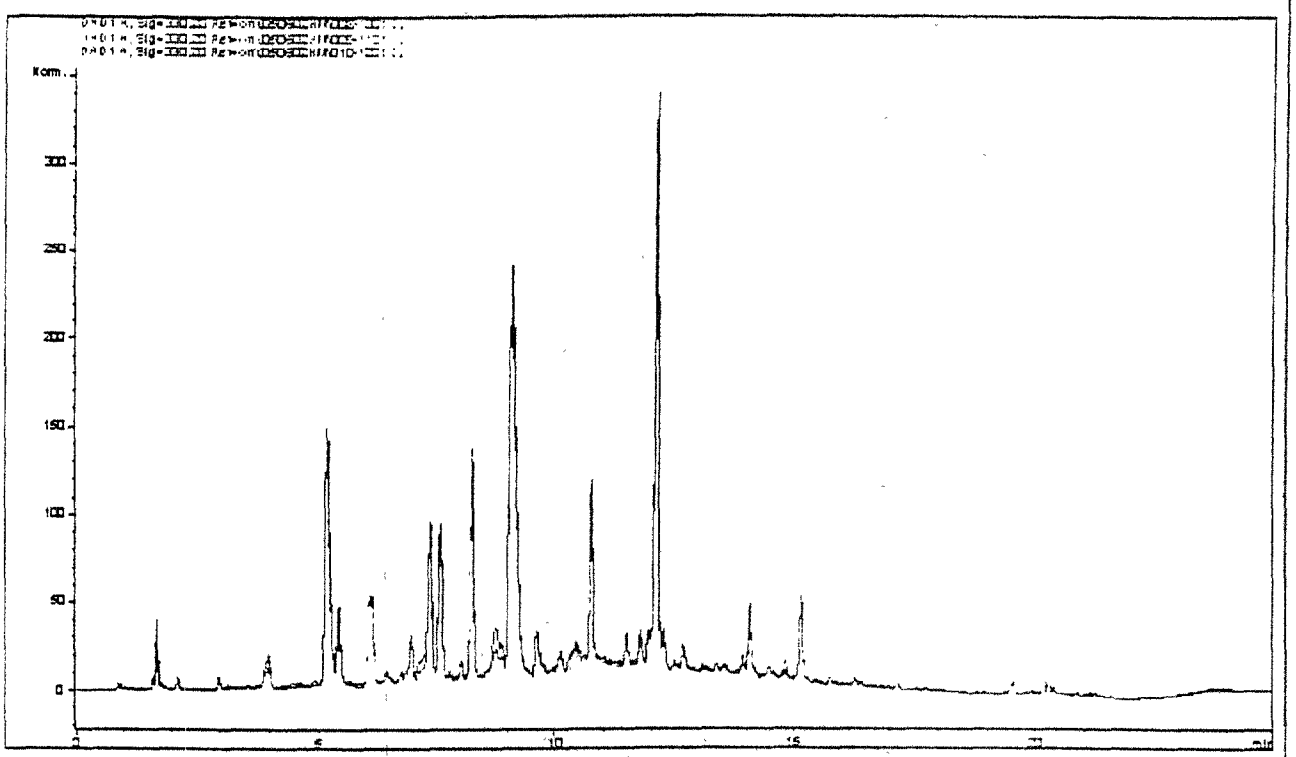
The HPLC profiles collected for the ACAPHA samples were subjected to a qualitative analysis. By integrating the major components in the HPLC profiles, the relative peak areas for major peaks were obtained. Through comparison of the retention time, and the relative retention time (relative to the most abundant peak in the botanical profile) the corresponding peaks in each profile were identified. By comparing the area abundances of these peaks we are able examine the differences between the different batches of the formulation. As peak area abundance is proportional to concentration for each peak, it is possible to compare the concentrations of chemicals extracted by the aqueous ethanol solution by the peak area abundances.

Results of HPLC Profile Analysis

ACAPHA 292 (Tag number 345)

Overlaid HPLC-DAD profiles for Within-Batch Chromatographic Comparison of ACAPHA 292-1, 292-2, 292-3

HPLC-DAD PROFILING OF ACAPHA 292 (Tag number 345)
292-1 (blue), 292-2 (red), 292-3 (green)



Calculated Data for Within-Batch Consistency for *ACAPHA 292 (Tag number 345)*

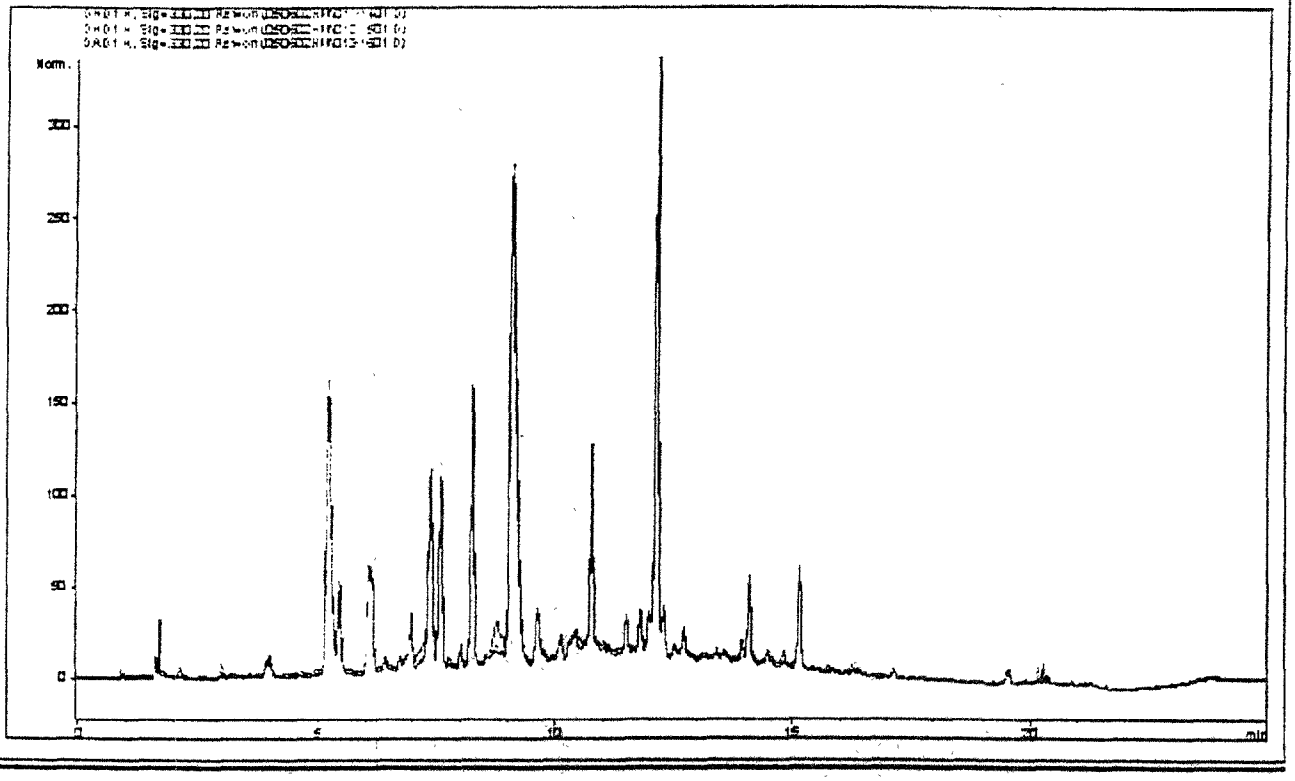
ACAPHA-292 (Tag 345)

Peak #	Retention time (min)	Relative Retention	Peak Area Abundance 1	2	3	mean	stdev	%RSD	Relative Peak Area Abundance (%) 1	2	3	mean	stdev	%RSD
1	1.61	0.18	24.70	23.55	16.07	21.48	4.71	21.95	0.24	0.22	0.16	0.21	0.04	18.77
2	1.69	0.19	58.09	60.79	94.00	70.96	20.00	28.18	0.56	0.58	0.96	0.70	0.23	32.44
3	3.99	0.44	140.86	141.51	167.91	150.10	15.43	10.28	1.35	1.34	1.71	1.47	0.21	14.36
4	5.19	0.57	1018.90	1037.35	942.32	999.52	50.39	5.04	9.78	9.84	9.61	9.75	0.12	1.22
5	5.42	0.60	245.55	240.32	224.36	236.74	11.04	4.66	2.36	2.28	2.29	2.31	0.04	1.83
6	6.07	0.67	233.28	233.42	226.45	231.05	3.98	1.72	2.24	2.21	2.31	2.25	0.05	2.20
7	6.13	0.67	215.39	221.93	188.67	208.67	17.62	8.45	2.07	2.11	1.92	2.03	0.10	4.70
8	6.97	0.77	158.43	161.60	156.59	158.87	2.53	1.59	1.52	1.53	1.60	1.55	0.04	2.65
9	7.38	0.81	614.41	620.34	581.39	605.38	20.99	3.47	5.90	5.89	5.93	5.91	0.02	0.39
10	7.60	0.84	518.57	523.59	494.23	512.26	15.85	3.09	4.98	4.97	5.04	5.00	0.04	0.77
11	8.27	0.91	684.24	694.59	631.65	670.16	33.75	5.04	6.57	6.59	6.44	6.53	0.08	1.21
12	9.09	1.00	2491.10	2537.69	2335.01	2454.60	106.15	4.32	23.92	24.08	23.82	23.94	0.13	0.54
13	9.64	1.06	240.32	242.11	243.09	241.84	1.40	0.58	2.31	2.30	2.48	2.36	0.10	4.35
14	10.76	1.18	621.73	627.63	586.12	611.89	22.53	3.68	5.97	5.95	5.98	5.97	0.01	0.19
15	11.48	1.26	188.49	189.63	182.10	186.81	4.14	2.21	1.81	1.80	1.86	1.82	0.03	1.67
16	11.78	1.30	219.09	218.76	209.94	215.93	5.19	2.40	2.10	2.08	2.14	2.11	0.03	1.58
17	11.96	1.32	199.76	197.23	185.04	194.01	7.87	4.06	1.92	1.87	1.89	1.89	0.02	1.25
18	12.12	1.33	1683.17	1698.19	1523.35	1634.90	96.50	5.93	16.16	16.11	15.54	15.94	0.34	2.16
19	12.28	1.35	191.53	194.14	182.62	189.43	6.04	3.19	1.84	1.84	1.86	1.85	0.01	0.71
20	13.92	1.53	98.77	99.53	93.22	97.17	3.44	3.54	0.95	0.94	0.95	0.95	0.00	0.35
21	14.07	1.55	255.09	255.47	225.59	245.38	17.14	6.99	2.45	2.42	2.30	2.39	0.08	3.30
22	15.15	1.67	314.73	319.61	313.03	315.79	3.41	1.08	3.02	3.03	3.19	3.08	0.10	3.12
			10416.20	10539.69	9802.75	10252.55			100.00	100.00	100.00			

ACAPHA 293 (Tag number 346)

Overlaid HPLC-DAD profiles for Within-Batch Chromatographic Comparison of ACAPHA 293-1, 293-2, 293-3

HPLC-DAD PROFILING OF ACAPHA 293 (Tag number 346)
293-1 (blue), 293-2 (red), 293-3 (green)



Calculated Data for Within-Batch Consistency for *ACAPHA 293 (Tag number 346)*

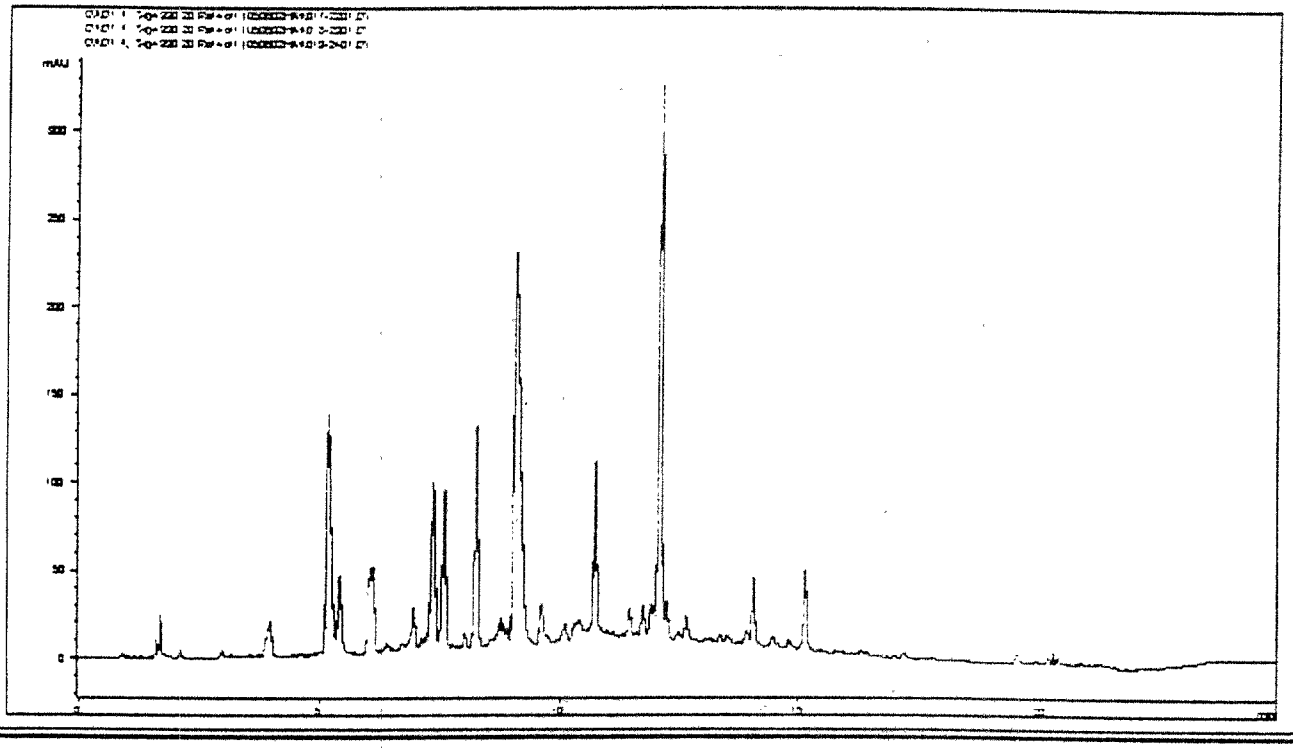
ACAPHA-293 (Tag 346)

Peak #	Retention time (min)	Relative Retention	Peak Area Abundance 1	2	3	mean	stdev	%RSD	Relative Peak Area Abundance (%) 1	2	3	mean	stdev	%RSD
1	1.61	0.18	19.65	34.65	36.68	30.33	9.30	30.68	0.19	0.31	0.31	0.27	0.07	24.33
2	1.70	0.19	67.10	35.77	26.85	43.24	21.14	48.89	0.66	0.32	0.23	0.40	0.23	57.36
3	3.99	0.44	109.91	102.36	101.12	104.13	4.19	4.02	1.07	0.90	0.85	0.94	0.12	12.40
4	5.19	0.57	989.40	1101.26	1139.80	1076.82	78.12	7.25	9.74	9.73	9.56	9.68	0.10	1.06
5	5.43	0.60	268.08	296.45	317.57	294.03	24.83	8.45	2.64	2.62	2.66	2.64	0.02	0.85
6	6.07	0.67	237.35	278.97	293.68	270.00	29.21	10.82	2.34	2.46	2.46	2.42	0.07	3.01
7	6.13	0.67	212.15	268.75	274.73	251.87	34.54	13.71	2.09	2.37	2.30	2.26	0.15	6.58
8	6.98	0.77	175.24	177.76	218.36	190.46	24.20	12.70	1.73	1.57	1.83	1.71	0.13	7.68
9	7.38	0.81	627.37	747.39	746.34	707.04	68.99	9.76	6.18	6.60	6.26	6.35	0.23	3.55
10	7.60	0.84	515.86	635.44	639.48	596.93	70.24	11.77	5.08	5.61	5.36	5.36	0.27	4.99
11	8.28	0.91	666.94	779.61	823.00	756.52	80.56	10.65	6.57	6.89	6.90	6.79	0.19	2.78
12	9.09	1.00	2436.33	2629.95	2825.47	2630.58	194.57	7.40	23.99	23.23	23.70	23.64	0.38	1.62
13	9.64	1.06	234.27	248.06	260.02	247.45	12.89	5.21	2.31	2.19	2.18	2.23	0.07	3.15
14	10.76	1.18	579.55	632.53	687.17	633.08	53.81	8.50	5.71	5.59	5.76	5.69	0.09	1.58
15	11.49	1.26	189.81	201.86	205.59	199.08	8.25	4.14	1.87	1.78	1.72	1.79	0.07	4.07
16	11.78	1.30	213.49	225.40	230.32	223.07	8.66	3.88	2.10	1.99	1.93	2.01	0.09	4.31
17	11.97	1.32	192.35	192.44	189.91	191.57	8.81	4.60	1.80	1.70	1.58	1.72	0.06	3.66
18	12.13	1.33	1598.30	1816.29	1929.90	1781.50	168.52	9.46	15.74	16.05	16.19	15.99	0.23	1.43
19	12.28	1.35	191.19	208.52	217.31	205.67	13.30	6.47	1.88	1.84	1.82	1.85	0.03	1.69
20	13.93	1.53	109.73	109.01	110.05	109.61	0.54	0.49	1.08	0.96	0.92	0.99	0.08	8.32
21	14.08	1.55	248.99	282.53	297.58	276.37	24.88	9.00	2.45	2.50	2.50	2.48	0.03	1.02
22	15.16	1.67	282.27	314.88	342.29	313.18	30.00	9.59	2.78	2.78	2.87	2.81	0.05	1.84
			10154.47	11319.90	11923.24	11132.54			100.00	100.00	100.00			

ACAPHA 296 (Tag number 349)

Overlaid HPLC-DAD profiles for Within-Batch Chromatographic Comparison of ACAPHA 296-1, 296-2, 296-3

HPLC-DAD PROFILING OF ACAPHA 296 (Tag number 349)
296-1 (blue), 296-2 (red), 296-3 (green)



Data for Within-Batch Consistency for *ACAPHA 296 (Tag 349)*

ACAPHA-296 (Tag 349)

Peak #	Retention time (min)	Relative Retention	Peak Area Abundance 1	2	3	mean	stdev	%RSD	Relative Peak Area Abundance (%) 1	2	3	mean	stdev	%RSD
1	1.61	0.18	42.73	41.44	46.23	43.49	2.51	5.78	0.38	0.37	0.39	0.38	0.01	2.21
2	1.70	0.19	56.22	59.52	52.29	56.01	3.62	6.47	0.50	0.53	0.44	0.49	0.05	9.92
3	3.99	0.44	138.42	141.17	137.53	139.04	1.90	1.37	1.24	1.26	1.15	1.22	0.06	4.86
4	5.18	0.57	1068.30	1041.62	1101.31	1070.41	29.93	2.79	9.54	9.31	9.20	9.35	0.17	1.84
5	5.42	0.60	305.79	314.54	327.22	315.85	10.78	3.41	2.73	2.81	2.73	2.76	0.05	1.66
6	6.07	0.67	278.87	269.06	295.17	281.03	13.19	4.69	2.49	2.40	2.47	2.45	0.04	1.79
7	6.13	0.68	269.85	279.15	295.95	281.65	13.23	4.70	2.41	2.50	2.47	2.46	0.04	1.81
8	6.98	0.77	197.64	198.14	208.39	201.39	6.07	3.01	1.76	1.77	1.74	1.76	0.02	0.89
9	7.38	0.81	670.54	666.94	725.23	687.57	32.66	4.75	5.99	5.95	6.06	6.00	0.05	0.85
10	7.60	0.84	584.53	581.12	630.79	598.81	27.74	4.63	5.22	5.19	5.27	5.23	0.04	0.74
11	8.28	0.91	733.79	734.38	820.07	762.75	49.64	6.51	6.55	6.56	6.85	6.66	0.17	2.55
12	9.07	1.00	2587.33	2595.65	2824.93	2669.63	134.57	5.04	23.10	23.21	23.60	23.30	0.26	1.13
13	9.63	1.06	289.29	288.04	331.54	302.95	24.76	8.17	2.58	2.57	2.77	2.64	0.11	4.18
14	10.76	1.19	671.87	667.48	703.25	680.87	19.51	2.86	6.00	5.97	5.88	5.95	0.06	1.07
15	11.48	1.27	225.37	225.41	225.03	225.27	0.21	0.09	2.01	2.01	1.88	1.97	0.08	3.91
16	11.77	1.30	238.82	238.80	236.63	238.08	1.26	0.53	2.13	2.13	1.98	2.08	0.09	4.33
17	11.96	1.32	212.30	211.02	215.06	212.79	2.07	0.97	1.90	1.89	1.80	1.86	0.05	2.93
18	12.13	1.34	1626.44	1624.12	1771.01	1673.86	84.19	5.03	14.52	14.52	14.80	14.61	0.16	1.10
19	12.28	1.35	243.49	243.60	239.45	242.18	2.36	0.98	2.17	2.18	2.00	2.12	0.10	4.77
20	13.93	1.53	125.09	125.56	117.13	122.59	4.74	3.86	1.12	1.12	0.98	1.07	0.08	7.59
21	14.08	1.55	318.91	317.97	329.85	322.24	6.61	2.05	2.85	2.84	2.76	2.82	0.05	1.82
22	15.15	1.67	314.44	322.23	334.44	323.70	10.08	3.11	2.81	2.88	2.79	2.83	0.05	1.64
			11200.05	11197.95	11958.56	11452.19			100.00	100.00	100.00			

FORENSIC SCIENCE CENTRE

Herbal Evaluation and Analysis Laboratory

October 2001 ***HPLC Profiling of ACAPHA Powders***

Collaborators *Dr. Wah Jun Tze, Dr. Joseph Tai, Dr. Stephen Lam*

Sampling Information & Testing Protocol

Nine ACAPHA powders were received from Dr. Joseph Tai by Ryan Takahashi for the Herbal Evaluation & Analysis Laboratory of the Forensic Science Centre. Sample log-in details, including the corresponding sample tag IDs, are described in Appendix A (Sample Receipt and Log-In).

All sample preparation and instrumental analysis was conducted by Ryan Takahashi under the supervision of Paula Brown, Principal Scientist, Forensic Science Centre, BCIT. The details of the sample preparation and HPLC methodology are as per the preliminary reports of January 2001 and February 2001.

Analysis of HPLC Profiles

The HPLC profiles of all the data collected were subjected to a qualitative analysis. By integrating the major components in the HPLC profiles the relative peak areas for major peaks were obtained. By comparing these peak areas we are able examine the differences between the different batches of the formulation. As peak area abundance is proportional to concentration for each peak, it is possible to compare the concentrations of chemicals extracted by the aqueous ethanol solution by the peak area abundances.

Within-Batch Consistency of Raw Botanicals

Triplicate aliquots of each powder sample were separately extracted and analysed by HPLC-DAD. The relative area abundance of the major peaks that were observed at 330 nm was compared to give an indication of the consistency of the plant material.

For all samples there existed a high level of consistency within-batch (less than 5% relative standard deviation for all peaks that account for more than 2% relative peak area). This consistency was comparable to the levels of deviation that have been observed in the previously analysed formulation samples. It has been previously reported that this level of deviation suggests that the sample is homogeneous.

Batch-to-Batch Consistency of ACAPHA powders

The batch-to-batch consistency of the powder samples was evaluated qualitatively. The analysis included overlaying the HPLC-DAD chromatograms obtained for representative samples of each ACAPHA powder, and the comparing the relative peak areas of major peaks observed when monitored at 330 nm for each powder. The percent relative standard deviation between the profiles gives an indication of the consistency of the raw botanicals.

In addition, a comparison of the batch-to-batch consistency for each herb was performed graphically with the relative abundances of peaks that eluted at the same retention time being compared. These charts are used as to represent the consistency of the material batch-to-batch, with batches that are consistent being represented by columns of a similar height at each retention time.

ACAPHA Powders (Tag numbers 213 to 221)

The nine samples that have been submitted for HPLC-DAD profiling show a high level of consistency between batches. The relative area abundances of the major peaks (>2% relative abundance) show a low level of deviation. Only three of the major peaks give a relative standard deviation of greater than 5%. These peaks elute at retention times of 3.98, 8.27, and 12.05 minutes, and show relative standard deviations of 39, 11, and 8%.

Two batches of ACAPHA powder show enhanced levels of the peak eluting at 3.98 minutes. Tag numbers 213 and 216 have relative abundances of this peak that are greater than the remaining batches.

A comparison of the relative peak area abundances for the major peaks observed in the nine batches of ACAPHA powders are shown as Appendix B.

The graphical representation of the relative peak area abundance comparison for batch-to-batch consistency is shown as Appendix C.

The chromatograms obtained for the ACAPHA powders when monitored at 330 and 260 nm are shown overlaid as Appendix D.

Appendix A: Sample Receipt & Log-In

Samples received from Dr. Joseph Tai on October 12, 2001

Received by: Ryan Takahashi
Description of samples: ACAPHA powders for HPLC-DAD profiling
Storage: room temperature
Condition of samples: good condition, sealed in individual plastic vials

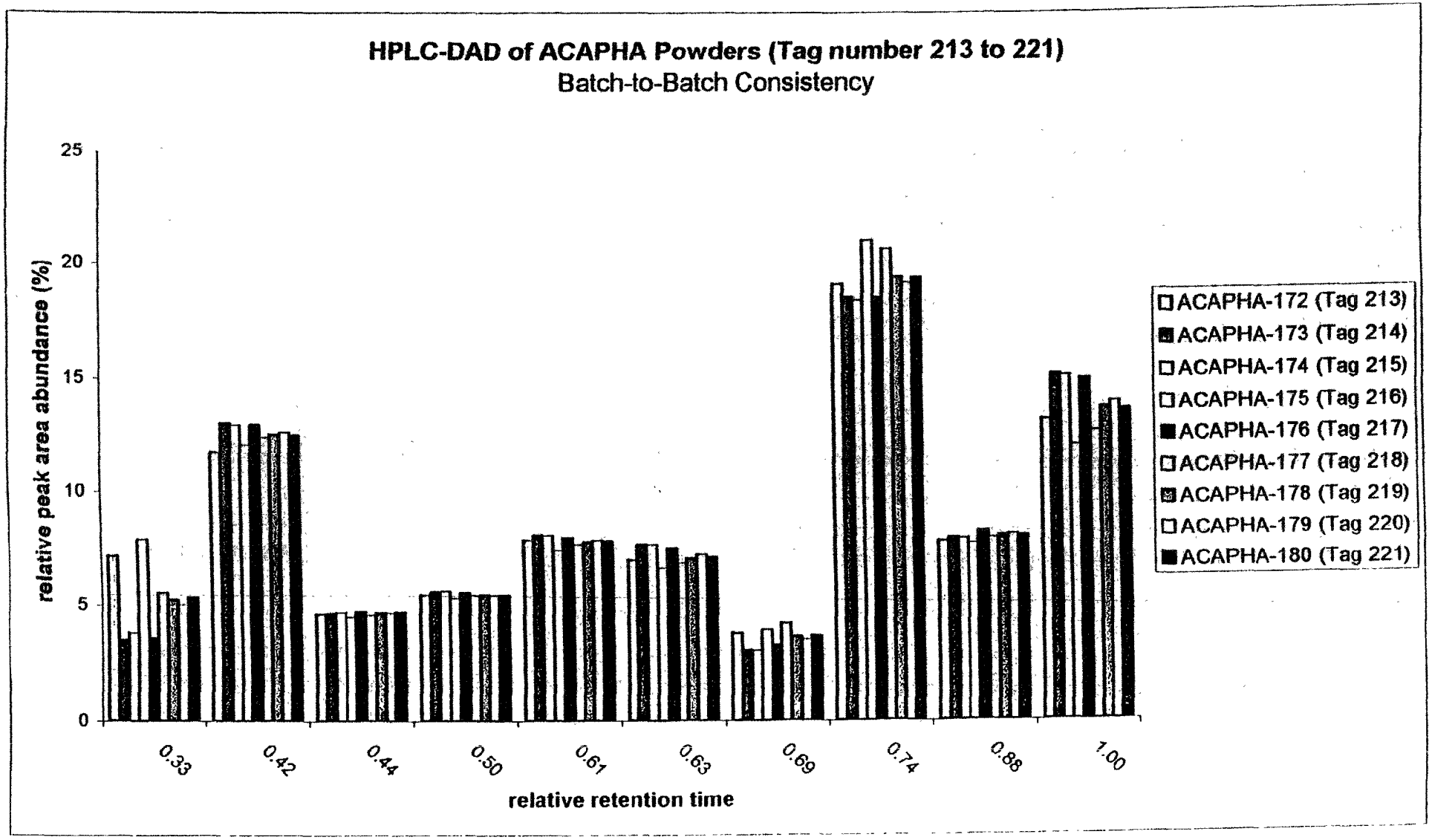
#	BCIT ID	Sample Identification		
		Date	Tag #	ACAPHA Sample*
1	ACAPHA-172	Oct 12 2001	213	Formulation 2 2000-1001 Bottle 1
2	ACAPHA-173	Oct 12 2001	214	Formulation 2 2000-1002 Bottle 1
3	ACAPHA-174	Oct 12 2001	215	Formulation 2 2000-1003 Bottle 1
4	ACAPHA-175	Oct 12 2001	216	Formulation 2 2000-1001 Bottle 2
5	ACAPHA-176	Oct 12 2001	217	Formulation 2 2000-1002 Bottle 2
6	ACAPHA-177	Oct 12 2001	218	Formulation 2 2000-1003 Bottle 2
7	ACAPHA-178	Oct 12 2001	219	Formulation 2 2000-1001+1002+1003
8	ACAPHA-179	Oct 12 2001	220	Formulation 2 2000-1001+1002+1003
9	ACAPHA-180	Oct 12 2001	221	Formulation 2 2000-1001+1002+1003

- 3 Lots of ACAPHA Formulation 2 Lot # 2000-1001, 2000-1002, 2000-1003 were sent to BCIT for HPLC profiling in May 2001. The samples were stored at room temperature and samples were resubmitted to BCIT for HPLC profiling on October 12 2001.
  2 samples removed from 2 different bottles of each lot were submitted. An additional 3 samples consisted of mixture of ACAPHA from the 3 lots.

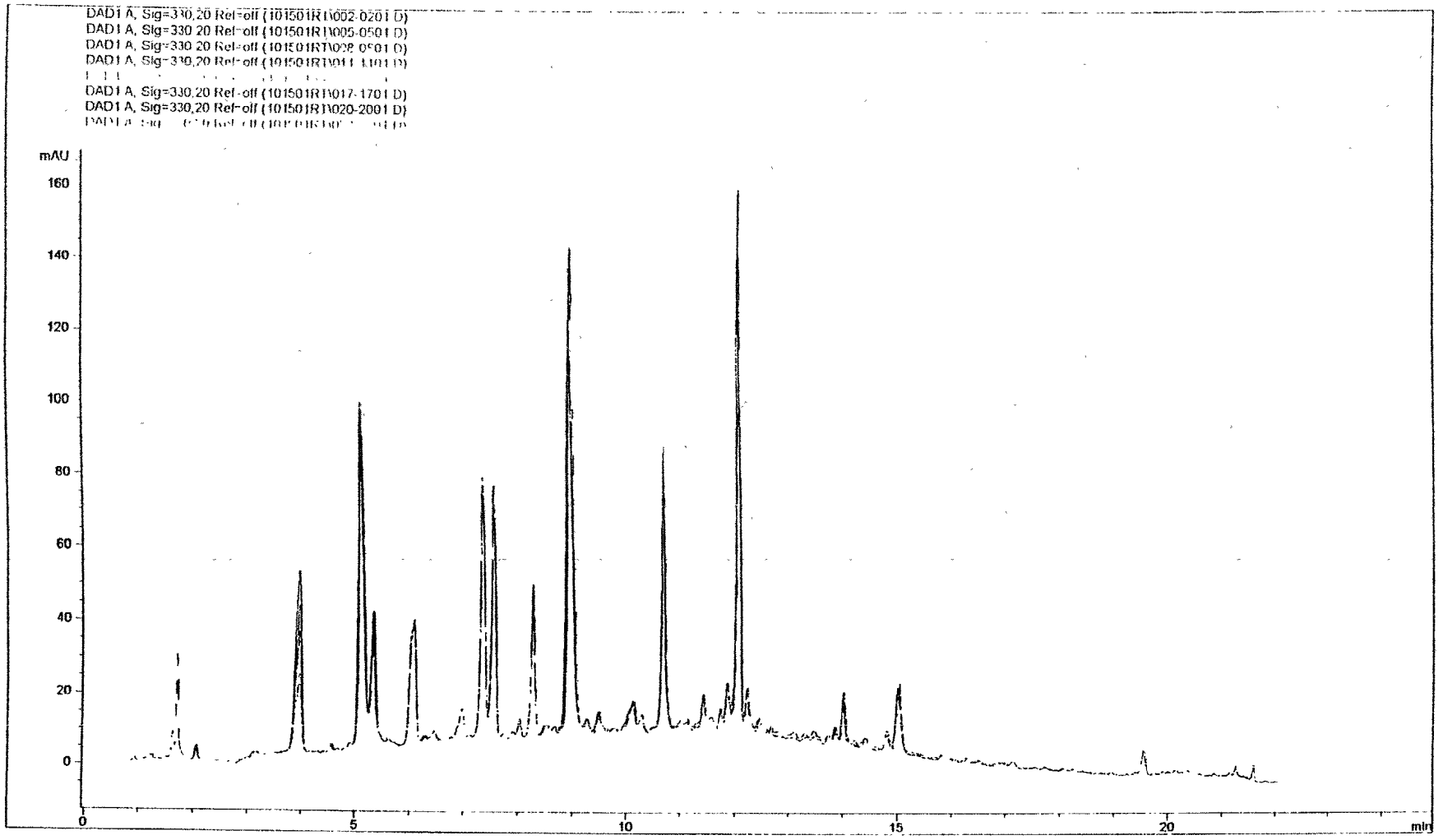
Appendix B: Batch-to-Batch Consistency of ACAPHA Powders

		Relative Peak Area Abundance									
	Retention time (min)	3.98	5.10	5.31	6.08	7.34	7.54	8.27	8.92	10.67	12.05
	Relative retention time	0.33	0.42	0.44	0.50	0.61	0.63	0.69	0.74	0.88	1.00
Sample	ACAPHA-172 (Tag 213)	7.14	11.74	4.60	5.45	7.81	6.96	3.79	19.11	7.80	13.17
	ACAPHA-173 (Tag 214)	3.49	13.04	4.63	5.58	8.06	7.61	3.04	18.53	7.96	15.19
	ACAPHA-174 (Tag 215)	3.81	12.93	4.68	5.60	8.04	7.62	3.04	18.39	7.90	15.10
	ACAPHA-175 (Tag 216)	7.83	12.07	4.47	5.30	7.38	6.60	3.91	21.03	7.69	12.05
	ACAPHA-176 (Tag 217)	3.59	12.95	4.72	5.53	7.94	7.46	3.25	18.49	8.25	14.98
	ACAPHA-177 (Tag 218)	5.53	12.39	4.56	5.42	7.64	6.82	4.21	20.62	7.94	12.69
	ACAPHA-178 (Tag 219)	5.24	12.52	4.68	5.45	7.74	7.05	3.63	19.40	8.07	13.73
	ACAPHA-179 (Tag 220)	5.00	12.60	4.65	5.43	7.81	7.20	3.50	19.17	8.10	13.98
	ACAPHA-180 (Tag 221)	5.33	12.46	4.70	5.43	7.76	7.10	3.65	19.39	8.05	13.65
	mean	5.22	12.52	4.63	5.46	7.80	7.16	3.56	19.35	7.97	13.84
	stdev	1.51	0.43	0.08	0.09	0.21	0.35	0.40	0.93	0.17	1.10
	%RSD	28.98	3.41	1.70	1.69	2.70	4.92	11.11	4.79	2.10	7.98

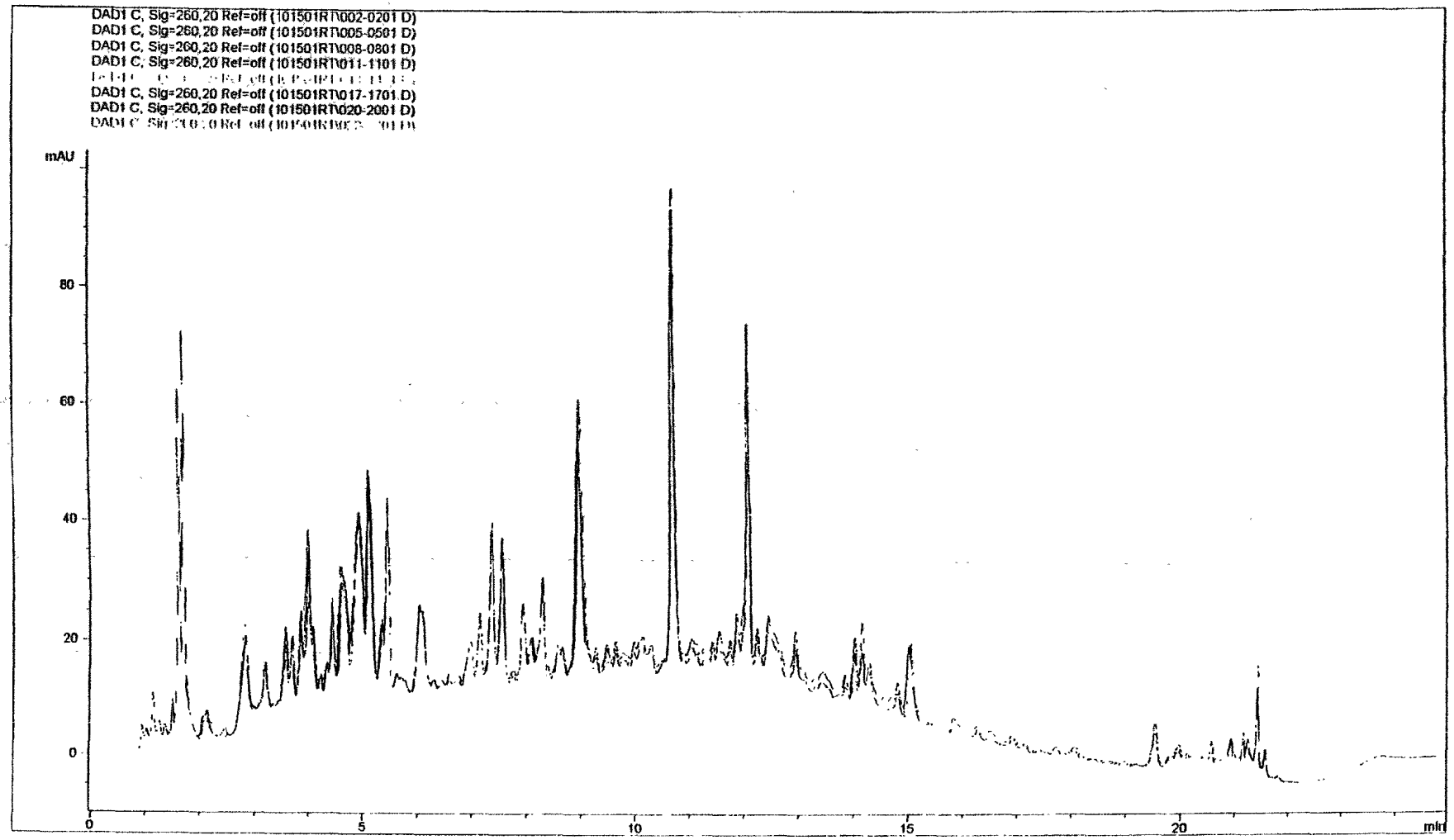
Appendix C: Batch-to-Batch Consistency of ACAPHA Powders



Appendix D: Batch-to-Batch Consistency of ACAPHA Powders (330 nm)



Appendix D *cont'd*: Batch-to-Batch Consistency of ACAPHA Powders (260 nm)



FORENSIC SCIENCE CENTRE

Herbal Evaluation and Analysis Laboratory

May 2001 *HPLC Profiling of ACAPHA Formulation 2*

Sampling Information

The following three samples were received from Dr. Joseph Tai,
Associate Professor, Department of Pathology University of British Columbia

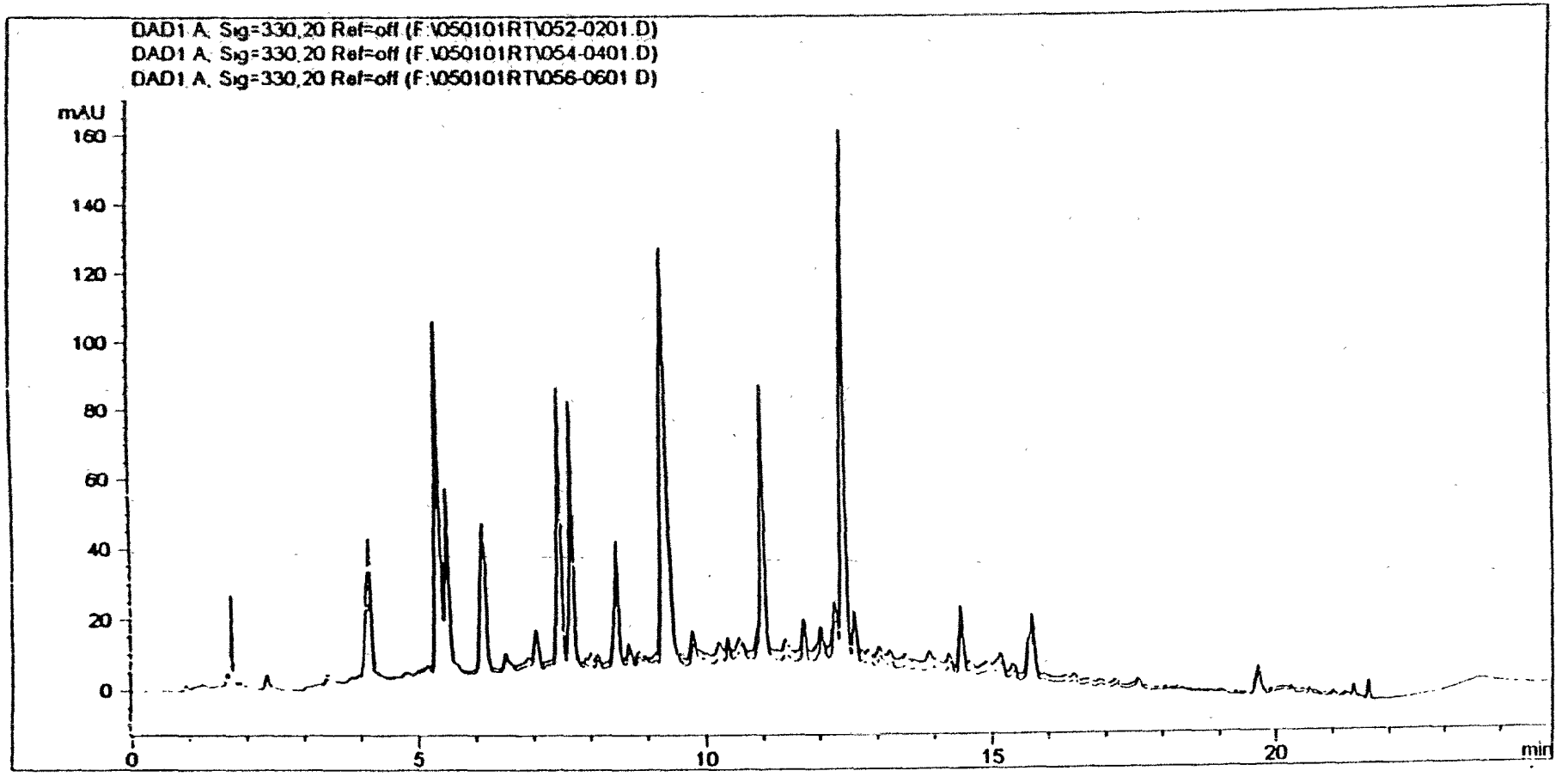
Samples: *Received by Ryan Takahashi*
Forensic Science Centre
British Columbia Institute of Technology

Sample	Lot #
ACAPHA Formulation 2	2000-1001
ACAPHA Formulation 2	2000-1002
ACAPHA Formulation 2	2000-1003

Testing Protocol

Three different batches of ACAPHA Formulation 2 were handed over to the Herbal Evaluation and Analysis Laboratory of the Forensic Science Centre. All sample preparation and instrumental analysis were conducted under the supervision of Paula Brown, Principal Scientist, Forensic Science Centre, BCIT. The details of the sample preparation and HPLC methodology are as per the preliminary reports of January 2001 and February 2001.

Formulation 2 (ACAPHA 2000-1001, 1002, 1003)
Batch-to-batch consistency, 330 nm

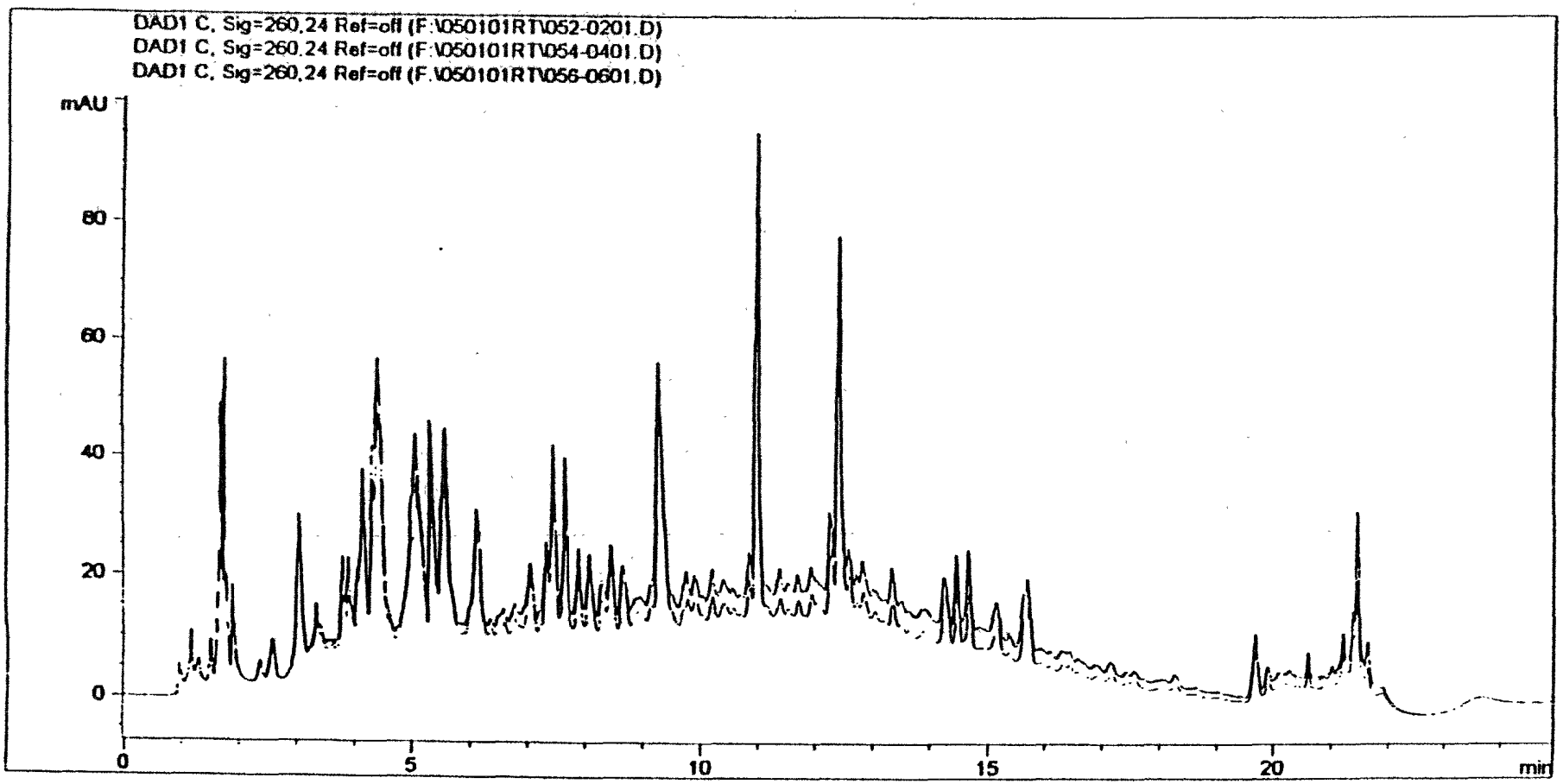


Appendix B

HPLC-DAD Profile of ACAPHA Formulation 2
(Samples ACAPHA 2000-1001, 2000-1002, 2000-1003)

ch-to-batch comparison

Peak #	Retention time (min)	Relative Peak Area Abundance (%)			mean	stdev	%RSD
		ACAPHA 2000-1001	ACAPHA 2000-1002	ACAPHA 2000-1003			
1	1.652	0.2675	0.2536	0.2462	0.2557	0.0108	4.2
2	1.741	1.6405	1.5350	1.5158	1.5638	0.0672	4.3
3	2.369	0.3992	0.3915	0.3604	0.3837	0.0205	5.3
4	4.147	6.2689	3.1626	4.0673	4.4996	1.5976	35.5
5	5.337	11.2611	12.2946	11.8401	11.7986	0.5180	4.4
6	5.503	5.3661	5.5323	5.5462	5.4815	0.1002	1.8
7	6.131	5.4333	5.5281	5.5322	5.4979	0.0560	1.0
8	6.512	0.4656	0.4804	0.5753	0.5071	0.0595	11.7
9	7.046	1.1099	0.9652	0.9649	1.0133	0.0836	8.3
10	7.441	7.6488	8.0493	7.9769	7.8916	0.2134	2.7
11	7.647	6.6813	7.3081	7.2291	7.0728	0.3414	4.8
12	8.435	3.9929	3.1045	2.9103	3.3359	0.5772	17.3
13	9.268	19.3804	18.3606	18.5511	18.7640	0.5422	2.9
14	10.991	7.3158	7.6087	7.5933	7.5059	0.1648	2.2
15	11.71	0.8629	0.8314	0.8115	0.8352	0.0259	3.1
16	12.026	1.0827	1.0426	1.0233	1.0495	0.0303	2.9
17	12.261	1.5354	1.7285	1.7961	1.6866	0.1353	8.0
18	12.409	12.6721	14.8730	14.4037	13.9829	1.1592	8.3
19	12.595	1.1537	1.1428	1.0778	1.1248	0.0410	3.6
20	14.463	1.4355	1.5045	1.7110	1.5503	0.1433	9.2
21	15.722	2.4259	2.5871	2.5036	2.5055	0.0806	3.2
22	17.57	0.3855	0.3162	0.3028	0.3348	0.0444	13.3
23	19.686	0.8586	0.9136	0.9625	0.9116	0.0520	5.7
24	21.371	0.1453	0.1697	0.1686	0.1612	0.0138	8.5
25	21.647	0.2118	0.3169	0.3307	0.2865	0.0650	22.7

* peaks that are >2% relative peak area abundance are highlighted in unshaded, bold typeface

Formulation 2 (ACAPHA 2000-1001, 1002, 1003)
Batch-to-batch consistency, 260 nm